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Fatty Acid Content in Shallow and Mesophotic Colonies of the Scleractinian Corals Montastraea cavernosa and Agaricia agaricites from the Cayman Islands

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Abstract

Two Scleractinian corals, *Agaricia agaricites* and *Montastraea cavernosa*, were collected from shallow (20 m depth) and mesophotic (60 m) ecosystems from the Cayman Islands. The fatty acid content of these species was analyzed by gas chromatography to determine variation in fatty acid profiles between the species and within species at different depths (shallow and mesophotic). Fatty acid composition is indicative of an organism's diet and can be used to determine feeding habits of corals. The corals studied had different fatty acid content, suggesting different feeding mechanisms. Additionally, a difference in fatty acid content arose as a result of living in different environments for *M. cavernosa* but not *A. agaricites*, suggesting feeding plasticity and supporting species-specific adaptations to new living environments.

In shallow ecosystems (20 m) *A. agaricites* contained more polyunsaturated fatty acids (PUFAs) than *M. cavernosa* and *M. cavernosa* contained more saturated fatty acids (SAFAs) than *A. agaricites*. There was no difference in the fatty acid profiles between colonies from mesophotic reefs at 60 m. The difference in FA content between species could be a result of species-specific feeding.

Shallow and mesophotic colonies of *A. agaricites* have similar fatty acid content while deep water colonies of *M. cavernosa* contain more PUFAs and less SAFAs than shallow colonies. This difference in fatty acid content may be a result of a species-specific response of these two organisms adapting to the different environments.

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Acknowledgments

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Introduction

Scleractinian corals are reef-building hard corals; the two Scleractinian corals in this study are *Montastrea cavernosa*, a head coral, and *Agaricia agaricites*, a plate coral (Figure 1). *M. cavernosa*, commonly known as the great star coral, is one of the most common reef-building corals of the Caribbean. It is known for its vibrant array of coloration including green, orange, brown, grey and red. While colonies are most abundant between 10 and 30 m, this organism has been documented as deep as 110 m (Arkive, 2015). *A. agaricites*, also known as the 'lettuce coral,' is another common Scleractinian species found from shallow environments to depths of 75 m (Aronson, Bruckner, Moore, Precht, & Weil, 2008).

There are many differences in shallow (1 - 30 m) and mesophotic (30 - 150 m) ecosystems. Mesophotic environments are understudied yet vitally important as they are thought to be a refuge for shallow water species as shallow water conditions become less favorable for coral growth as a result of changing ocean conditions and pollution (Lesser, Slattery, & Leichter, 2009). Despite this, numerous abundant shallow water species do not colonize mesophotic coral ecosystems (MCEs), showing that deep water does not serve as refuge for all coral species (Kahng et al., 2010). Scleractinian corals are abundant in MCEs which range from 30 m to 150 m (Rooney et al., 2010). The zooxanthellae taxa in mesophotic coral colonies can be different compared to shallow ecosystems where light and photosynthetically active radiation (PAR) levels are lower. Whether corals in these deeper waters alter their modes of nutrient acquisition from translocation of nutrients from their symbiotic zooxanthellae or an increase in heterotrophy is still uncertain (Kahng et al., 2010). Individual coral species utilize

different feeding strategies and thus no universal pattern of adaptation to depth can be identified, as the acclimation to depth is likely to be species-specific. Species-specific adaptations have been shown in relation to varying temperatures (Coles & Fadlallah, 1991), light levels, and food availability (Tremblay, Grover, Maguer, Hoogenboom, & Ferrier-Pagès, 2014). Depth, which may affect any of these factors, could also cause a species-specific response.

Corals feed by two mechanisms as corals exhibit polytrophism: that is they can attain nutrients via heterotrophy or by translocation of photosynthates by symbiotic zooxanthellae living within the coral tissue (Ayukai, 1995). Some coral species are shown to depend more on heterotrophy than translocation, this behavior has been documented for the corals in our study. *A. agaricites* is a heterotrophic feeder, while *M. cavernosa* relies more on translocation from symbionts for its nutrients (Crandall, Teece, Manfrino, Estes, & Ciesla, 2015). Factors such as light and food availability would cause our two corals to react differently as *M. cavernosa* is more dependent on higher PAR levels which are important for photosynthesis and thus translocation. The relative importance of heterotrophy and autotrophy is species-specific, with only certain corals exhibit heterotrophic plasticity (Anthony & Fabricius, 2000).

To determine differences in feeding habits between coral species, several methods can be used including comparisons of fatty acid profiles. Fatty acids are molecules used by cells for a variety of reasons including the construction of lipids, triglycerides, and other macromolecules. Generally, fatty acids can be categorized into three broad groups based on specific functions. Saturated fatty acids (SAFAs) consist of a fully saturated hydrocarbon chain; monounsaturated fatty acids (MUFAs) contain one double bond in

the alkyl chain, typically at or before the C9 position for organisms of *Animalia*; and polyunsaturated fatty acids (PUFAs) which have more than two double bonds in the alkyl chain (Gurr, Harwood, & Frayn, 2002).

Only plants and some bacterial organisms are known to synthesize PUFAs, which are essential to health, reproduction, and membrane fluidity of higher trophic level organisms (Spector, 1999) (Murata & Los, 1997). Typically, organisms of the kingdom *Animalia* must obtain these essential PUFAs through their diet. Corals are interesting organisms as they contain symbiotic algae in their tissues which can transfer these essential fatty acids (PUFAs) to the coral cells via translocation (Schlichter, 1982). In addition to this, corals can also feed heterotrophically to obtain PUFAs (Jakobsen, 1999). Symbiotic algae of corals can transfer enough lipids to the host to make up 90% of the host organisms lipid content (Muscatine, 1990).

However when introduced to environmental stressors a shift in feeding habit is often observed. Stressors studied include water temperature (Glynn, Perez, & Glichrist, 1985), food source availability (Imbs , Latyshev, Dautova, & Latypov, 2010), light intensity (Harland, Davies, & Fixter, 1992), and zooxanthellate photosynthetic productivity (Rodrigues, Grottoli, & Pease, 2008). In the coral *Stylophora pistillata*, photosynthetic rates and translocation of nutrients was observed under varying irradiance and food availability. Different percentages of lipids transferred to the coral by translocation were observed under the different irradiances and under different food availability conditions; 90% translocation was observed in colonies with high irradiance and high food availability while 71% translocation was observed in low irradiance and

high food availability (Tremblay et al., 2014). This study shows zooxanthellae performing differently when exposed to different light levels.

An extreme example of change in nutrient acquisition of corals in differing environments is coral bleaching, where increases in water temperature cause the coral tissue to be an ill-suited environment for the algae which then leave the coral tissue and the coral must obtain all its nutrients via heterotrophy (Glynn, 1993). As shifts in feeding mechanisms have been observed with different stressors, there is no universal cause and effect between these factors. It has been proposed that feeding mechanisms may be species-specific. Two corals studied *Goniastrea retiformis* and *Porties cylindrica* were exposed to different light levels and suspended particulate matter (SPM). Under controlled conditions the corals reacted differently to the varying light and SPM; *G. retiformis* was able to grow under high SPM while *P. cylindrica* became energy deficient (Houlbreque & Ferrier-Pages, 2009). This study by Houlbreque & Ferrier-Pages (2009) shows a species-specific response.

Another species-specific trait of corals is the type of symbiont (*Symbiodnium*) associated with each coral. For example, the corals *Porcillopora verrucosa* and *Pavona giganteca* contained different symbionts over a depth range which had adapted to different light regimes suggesting host-specific symbionts (Iglasias-Prieto, Beltran, LaJeunesse, Reyes-Bonillas, & Throme, 2004). Additionally, the types of *Symbiodinium* changed with increasing depth in *M. cavernosa* (Lesser et al., 2010). Variation in symbionts within coral tissue may play a major role in translocation and fatty acid content within corals.

M. cavernosa and *A. agaricites* are within different taxonomic families, express different morphologies (Figure 1), and favor one feeding mechanism over the other (translocation favored in *M. cavernosa*, heterotrophy favored in *A. agaricites*). It is hypothesized that the species will have different fatty acid content between each other because of those properties. In addition to this, it is hypothesized that the species will behave differently in shallow and mesophotic environments, demonstrating a species-specific response to environmental conditions that differ from shallow to mesophotic ecosystems such as light levels.

Such behavioral change can be seen in the fatty acid profile of the species and may cause the corals to shift feeding habits. It is for that reason that change is expected most in the PUFA content as PUFAs are attained in organisms from *Animalia* strictly through their diet. It is possible that the lower light levels in mesophotic environments will cause the symbiotic algae to be less productive (Harland et al., 1992) thus corals more dependent on nutrient acquisition from translocation would acquire less PUFA. However, the coral could obtain those PUFAs via heterotrophy which would result in no change in PUFA content or possibly an increase in PUFA depending on food availability and feeding rate.

Methods

Sample collection

Samples of *Montastraea cavernosa* and *Agaricia agaricites* were collected from the Cayman Islands. Trained technical divers collected coral chips at “Rock Bottom” (19° 41.67” N, 80° 4.17” W) and “Paul’s Anchors” (19° 42.0” N, 80° 3.42” W) located on the north wall near the Little Cayman Research Centre using SCUBA (Figure 1). Individual

colonies were sampled from a shallow zone at 18-20 m (*M. cavernosa* n=5; *A. agaricites* n=4) and from the mesophotic zone at 55-60 m (*M. cavernosa* n=5; *A. agaricites* n=4). Samples were packed in labeled vials and returned promptly to the laboratory where the top 4mm of coral holobiont (animal and symbiont) tissue was scraped from each colony and then stored in a freezer. Frozen samples of the holobioint (animal tissue, symbiont, and microbial consortia) were ground with a mortar pestle to homogenize samples to a powdery consistency. This powder was lyophilized (-70°C, 150 mTorr) for 48 hours to remove residual water.

Fatty acid analysis

Total organic matter was extracted from approximately 40 mg of dry, homogenized coral tissue. The tissue homogenate included the host animal tissue, the symbionts, and associated biomass. Organic matter was extracted using dichloromethane (DCM) and methyl alcohol (1:1). 2 mL of the solvent mixture was added to the weighed tissue sample in pre-muffled (400°C, 2 hr) test tubes and vortex mixed. The mixed sample was sonicated in a water bath for 5 minutes and centrifuged (4g, 2 min) to form a solid pellet. The supernatant was removed with new pre-muffled Pasteur pipettes to a fresh pre-muffled test tube. The extraction and removal of the supernatant was performed 2 more times and the solvent extract fractions were combined. The extract was dried at 50°C under a gentle stream of nitrogen. After drying, tricosanoic acid was added to the extract as an internal quantitation standard and dried under a stream of nitrogen at 50°C.

Total organic extracts were saponified (6 % potassium hydroxide in methanol for 60 minutes at 70 ^{o}C) to isolate fatty acids and subsequently esterified with methanolic hydrochloric acid (Sigma, 60 minutes at 60 ^{o}C), and free alcohols silylated with

bis(trimethylsilyl)-trifluoroacetamide (BSTFA; Sigma; 60 minutes at 60 ^{o}C). Gas chromatography (GC) analysis was performed on a Hewlett Packard 5890 using a DB-5 (J&W Scientific) column (30 m, ID 0.25 mm, film thickness 0.25 mm). The column temperature program started at 60 ^{o}C for 1 minute, increased to 140 ^{o}C at 15 ^{o}C min^{-1}, and then increased to 300 ^{o}C at 4 ^{o}C min^{-1} and remained there for 15 minutes. The split/splitless injector and detector temperatures were both 250 ^{o}C, and the column flow was 2.6 ml min^{-1} of helium.

Fatty acids were identified by comparison with known fatty acids (Sigma-Aldrich) and by high-resolution mass spectrometry (ThermoFinnigan Trace GC Ultra coupled to a high resolution ThermoFinnigan MAT 95 XP MS [GC-MS]) operating in electron-impact mode with the column and temperature program described above. Mass spectra, equivalent chain length measurements (ECL; Christie 1988) and relative retention times were compared with known standards and published spectra. Quantitation of fatty acids was determined by the FID chromatogram using the internal standard (tricosanoic acid), and are expressed as percentage of total fatty acids. The instrument detection limit was 2 ng on the GC column, and the error in measurement for each compound was <5%.

Statistical analysis

Fatty acid percentage data were normalized via arcsine transformation (Zar 1984), and fatty acid amounts were individually compared between the two species using a 2-sample T-test. We also tested whether individual species of corals contained higher relative abundances of specific fatty acids between shallow and deep colonies using a

one-sample t-test. All statistical tests were performed using MiniTab Release 17 software and comparisons were considered significant at $\alpha = 0.05$.

Results

Scleractinian coral comparison of FA content between species

The average fatty acid profiles of *A. agaricites* and *M. cavernosa* were tabulated (Table 1). Values for relative abundance of each fatty acid was calculated as percent of average total fatty acid content. To calculate average SAFA, MUFA, and PUFA content for each species; the average content of the fatty acids C14:0, C16:0, and C18:0 (SAFA); C16:1 ω 9, C16:1 ω 7, C18:1 ω 9, and C18:1 ω 7 (MUFA); and C18:2 ω 6, C20:4 ω 6, C20:5 ω 3, C20:3 ω 6 , C22:6 ω 3, C22:4 ω 6, C24:5 ω 6, C24:6 ω 3 (PUFA) were combined and normalized to the total FA content of the samples. *M. cavernosa* and *A. agaricites* contained similar fatty acid compounds including saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

For both species, SAFA was the dominant fatty acid class making up 71-80% total FA content in *M. cavernosa* and 64-72% in *A. agaricites* (Table 1, Figure 4). The dominant fatty acid for both species was C16:0 (55-60% *M. cavernosa*; 41-57% *A. agaricites*) followed by C18:0 (13-18% *M. cavernosa*; 11-21% *A. agaricites* (Table 1, Figure 3).

The ω7 and ω9 monounsaturated C16 and C18 moieties were present in less than 2.2% in *M. cavernosa* (with the exception of C18:1ω9 which amounted to as much as 8%) and 2.6% in *A. agaricites* (with the exception of the C18:1ω9 which amounted to as much as 7.1%). Combined MUFAs accounted for up to 12% of the fatty acid content in

both species at all depths. Total PUFAs accounted for up 25% of the total fatty acid content in shallow colonies of *A. agaricites* and were dominated by 22:6ω3 (up to 6.5% in *M. cavernosa* and 6.4% in *A. agaricites*) (Table 1).

Shallow colonies of *A. agaricites* and *M. cavernosa* varied in SAFA and PUFA content (Figure 4a). *M. cavernosa* had higher SAFA content [$t(5)=5.38$ ($p=0.003$)], and lower PUFA content [$t(5)=4.89$ ($p=0.005$)] than *A. agaricites*. Additionally, in shallow colonies there was no difference in MUFA content between species [$t(3)=1.20$ ($p=0.316$)]

Mesophotic colonies (60 m) showed no difference in SAFA [$t(12)=0.34$ ($p=0.738$)], PUFA [$t(13)=0.25$ ($p=0.809$)], or MUFA [$t(10)=0.78$ ($p=0.454$)] (Figure 4b).

Scleractinian coral response to depth

The major fatty acid classes (SAFA, MUFA, PUFA) between shallow (20 m) and mesophotic (60 m) colonies of *A. agaricites* were similar (Figure 5b; SAFA: [$t(9)=1.54$ ($p=0.158$)], MUFA[$t(5)=0.38$ ($p=0.722$)], PUFA: [$t(9)=1.65$ ($p=0.134$)].

In shallow and mesophotic colonies of *M. cavernosa*, there were differences in overall SAFA and PUFA content (Figure 5a). Shallow colonies contained more SAFA: [$t(11)=2.62$ ($p=0.024$)]; while deep colonies contained more PUFA: [$t(12)=0.34$ ($p=0.025$)]. There was no difference in MUFA content between the two depths [$t(11)=0.55$ ($p=0.596$)].

Discussion

Comparison of scleractinian coral fatty acid content

We compared the fatty acid content of the Scleractinian corals *M. cavernosa* and *A. agaricites*. As expected, shallow water colonies of *M. cavernosa* and *A. agaricites*

have different fatty acid content particularly in the SAFA and PUFA. However, mesophotic colonies contained similar fatty acid compositions (Table 1).

Of the shallow colony data, *A. agaricites* contain more PUFA than *M. cavernosa* while *M. cavernosa* colonies contain more SAFA than *A. agaricites*. This variation in our Scleractinian coral FA content complies with the literature where FA content varies between species of Scleractinian coral with no correlation to genus or morphology. In a study of 11 species of Scleractinian corals of various genera and morphology, no correlation of fatty acid content to morphology or genera was observed (Yamashiro, Oku, Higa, Chinen, & Sakai, 1999). As organisms occupy various ecosystem niches they adapt different characteristics, one being morphology. This is a result of evolution, a well known example is how Darwin's finches took on different beak sizes as they fed on different sources and occupied different niches in the Galapagos (Grant, 1999). This is the basis of evolution, corals could simply gain different morphologies as a result of competition leading to species-specific developments in FA content and morphology.

In an additional study, the fatty acid content of sixteen Scleractinian species belonging to six families varied between families (A. B. Imbs, Demidkova, Latypov, & Pham, 2007). As *M. cavernosa* is a member of the *Faviidae* family, and *A. agaricites* is a member of the *Agariciidae* family; these species would likely have different fatty acid content based on the Imbs (2007) results which supports possible chemotaxonomic abilities to place corals in a phylogenetic tree. In our study, there were only variations between the shallow water colonies of *A. agaricites* and *M. cavernosa* while the mesophotic colonies had comparable fatty acid content. Imbs et al. (2007) only analyzed colonies growing in shallow water (2 m) and reported variation between families where

conditions for photosynthesis are favorable for the zooxanthellae. In our study, corals of two families, *M. cavernosa* and *A. agaricites*, collected in shallow waters had varying FA content while mesophotic colonies had similar FA content. *M. cavernosa* is a more photosynthate-dependent organism than *A. agaricites*, which is a substantial heterotrophic feeder (Crandall et al., 2015). As *M. cavernosa* colonizes deeper environments where photosynthesis may be reduced as a result of lower PAR levels, it may have to adapt to rely more on heterotrophy than shallow colonies which is why a change in FA content was observed. For *A. agaricites* no change in FA content was observed as it is already a heavy heterotrophic feeder.

Individual coral species may have different feeding capabilities. For example, the coral *Pavona gigantean* has a higher feeding rate than *Pocillopora damicornis* (Palardy, Grottoli, & Matthews, 2006), (Palardy, Grottoli, & Matthews, 2005). Because PUFAs are brought into the organism through their diet, corals with the ability to feed faster will have more PUFA content as they can obtain food at an accelerated rate. It is possible that *M. cavernosa* and *A. agaricites* simply have different feeding rates, which could be a result of their differing morphologies. *A. agaricites* is a heavier heterotrophic feeder than *M. cavernosa* (Crandall et al., 2015) which could account for the variation in FA content of shallow colonies as conditions for photosynthesis enable *M. cavernosa* to obtain nutrients including fatty acids mainly via translocation. The two coral species can feed by different mechanisms in shallow environments as there are optimal conditions for photosynthesis and heterotrophy, leading to variation in FA content as FA content relies on an organism's diet. In mesophotic environments, however, photosynthesis is inhibited thus the coral that relies more on translocation may need to adapt to more heterotrophic

feeding which can be inferred by a change in diet between shallow and deep colonies leading to a change in the FA content.

Scleractinian colony depth comparison

In our study, shallow and mesophotic colonies of *M. cavernosa* and *A. agaricites* were collected and compared in terms of their fatty acid content to observe differences in fatty acid content in different water environments. It was expected that shallow and deeper colonies of these corals would have different fatty acid content based on the different environmental conditions imposed by increasing depth including temperature and photon flux density. We hypothesized that the coral species would behave differently in relation to depth. Indeed, shallow and mesophotic colonies of *M. cavernosa* have different fatty acid content while shallow and mesophotic colonies of *A. agaricites* have comparable fatty acid content. This shows that the corals do in fact behave differently in shallow and mesophotic environments compared to each other. This could be due to the feeding habits of these specific corals, as *M. cavernosa* is more dependent on its photosynthetic zooxanthellae than *A. agaricites* which relies primarily on heterotrophy for nutrients (Crandall et al., 2015).

As environmental conditions change from shallow to mesophotic ecosystems, photosynthesis may be less productive causing a shift in feeding for *M. cavernosa*. Photosynthetic variation and increased heterotrophy with depth was observed for *M. cavernosa* (Lesser et al., 2010). Lesser (2010) showed that temperature change did not play a major role in shallow and mesophotic environments, only a 4°C difference was observed between shallow and mesophotic (90 m) environments suggesting that light or PAR availability was the determining factor for the changes seen in colonies of *M.*

cavernosa in shallow and mesophotic environments as light levels are known to vary with depth (Harland et al., 1992).

Shallow and deep colonies of *M. cavernosa* had different fatty acid content with deep colonies contained more PUFA and less SAFA content than shallow colonies (Figure 5a). As mesophotic colonies are exposed to less light, it would be expected that photosynthesis would slow and less translocation could take place between the symbionts and coral tissue resulting in a decrease in PUFA content as translocation is a main method by which some coral species obtain PUFAs. Instead, we observed an increase in PUFA content in *M. cavernosa* mesophotic colonies compared to shallow colonies (Figure 5a, Table 1). A possible explanation as to why PUFA content increased with increasing depth is an increase in heterotrophic feeding in mesophotic colonies, as shown by Lesser et al. (2010): *M. cavernosa* exhibited a sharp transition from translocation to heterotrophy between colonies collected at 45 and 61 m. In some corals, such as *Pocillopora damicornis*, feeding rate is dependent on photosynthate availability; that is, as the symbionts are unable to photosynthesize or photosynthetic rates decrease, heterotrophy will increase (Clayton Jr. & Lasker, 1982). This switch in feeding mechanism has been shown to be species-specific as some species of coral are more dependent on photosynthetic nutrients while others are mainly heterotrophs even in shallow environments (Crandall et al., 2015).

Zooxanthellae are present in deeper colonies as zooxanthellae within the coral tissues are able to undergo photo-acclimation to persist in environments of changing light (Titlyanov, Titlyanova, Yamazato, & van Woesik, 2001). The coral *Stylophora pistillata* can survive between 95% and 0.8% PAR_0 with 30% to 8% PAR_0 as the optimum light

harvesting region for the zooxanthellae (Titlyanov et al., 2001). Changes in chlorophyll and zooxanthellae population densities in coral branches were tested at different light levels to show productivity. It is possible that *M. cavernosa* was not outside it's optimal PAR range as well because the coral did not appear to be 'stressed;' PUFA content of the coral actually increased. Because PAR values were not recorded during our sample collection, there is no way to determine if this was the case. Comparison to other studies on the depth effects on *M. cavernosa* reveal that the coral is able to photoacclimate up to 91 m, with an apparent shift to heterotrophic feeding from 45 to 61 m (Lesser et al., 2010). The increased PUFA content in *M. cavernosa* with increasing depth within our study may be a result of increased heterotrophy given proper food availability.

Another experiment explored the effect of different light and food availability on the coral *Turbinaria reniformis.*. Lowest SAFA and PUFA production was observed in starved and low light colonies, whereas the highest PUFA production was in colonies grown under low light and fed conditions (Treignier, Grover, & Ferrier-Pages, 2008). The later results revealing highest PUFA production at low light agrees with our study as light is substantially lower in mesophotic environments compared to shallow environments. It is possible that an abundance of food was available for *M. cavernosa* to take in, resulting in an increase in PUFA despite the possible decreased potential to obtain these nutrients from translocation. However, for the Scleractinian coral *Stylophora pistillata*, there was no difference in capture rates between highly fed and slightly fed coral colonies (Ferrier-Pagés, Witting, Tambutté, & Sebens, 2003). It is possible that shallow and deep colonies of *M. cavernosa* had similar feeding opportunities, but the deep colonies fed more than the shallow colonies. Recent data has shown a link between photosynthesis and

heterotrophy. Translocation within the coral *Stylophora pistillata* was studied under varying levels of irradiance and food availability. The greatest amount of nutrients transferred to the coral via translocation was recorded to be 90% under high irradiance when fed while the lowest nutrient acquisition via translocation was 71% under low irradiance when fed (Tremblay et al., 2014). This brings back the dependence of coral heterotrophy on photosynthate availability where feeding rates have been shown to increase when photosynthetic activity decreases (Clayton Jr. & Lasker, 1982). While *M. cavernosa* may have had similar feeding opportunities in shallow and mesophotic environments, the lower light conditions in the mesophotic environment may have caused a decrease in photosynthetic activity and thus a decrease in nutrient transferred to the coral through translocation. This decrease in photosynthate availability would have caused the coral to increase heterotrophy, which would otherwise not have occurred if optimal light-levels were present for the zooxanthellae as shown in shallow colonies. This accounts for the variation in fatty acid content of *M. cavernosa* in shallow and mesophotic environments.

M. cavernosa is dependent on photosynthate nutrients while *A. agaricites* relies more on heterotrophy for nutrient acquisition (Crandall et al., 2015). This could explain why the fatty acid content in shallow and mesophotic colonies of *M. cavernosa* changed while that of *A. agaricites* did not. It was suggested that the decreased availability of photosynthate nutrients caused mesophotic colonies of *M. cavernosa* to increase heterotrophy compared to shallow colonies, which rely on zooxanthellae for nutrients. If *A. agaricites* does not rely heavily on photosynthate nutrients for survival, then there should be no change in fatty acid content when light levels change as *A. agaricites* is

naturally a substantial heterotrophic feeder. The difference between the corals *A. agaricites* and *M. cavernosa* supports the possibility that coral feeding habits are species-specific. For example, the feeding habits of individual colonies of the Scleractinian corals *Orbicella* (*Montastraea) faveolata* and *Porites astreoides* cannot be correlated to a specific factor (Teece, Estes, Gelsleichter, & Lirman, 2011). Teece et al. (2011) observed colonies under multiple conditions including turbidity, sedimentation, and nutrient levels. The different stressors caused the corals to have different fatty acid content but it was found that the coral feeding habits were variable in relation to the different conditions and thus there was no universal pattern of the coral feeding habit in relation to the specific stressors. The corals *Porites compressa* and *Montipora capitata* reacted differently when introduced to coral bleaching environments in terms of their lipid classes (Rodrigues et al., 2008), reinforcing species-specific reaction to stress of new environments. In addition to these stressors, the effect of suspended particulate matter (SPM) as a stressor or method of nutrient acquisition by corals has also been studied. Increased SPM in reef waters can affects light levels available to corals as the more turbid the water (high SPM) the less light the corals can get as the photons can not penetrate through the suspended particulate matter. A study observed the feeding abilities of *Goniastrea retiformis* and *Porites cylindrica* under increased SPM – decreased light and food potential within the SPM. Under high SPM content, *G. retiformis* increased feeding rates and tissue mass while *P. cylindrica* did not increase feeding and showed a loss of tissue (Anthony & Fabricius, 2000). The authors proposed that *P. cylindrica* had reached its 'feeding saturation' and, in an environment where particle saturation was above that of the corals feeding saturation, a stress response was triggered and the coral lost energy. Studies such

as Anthony & Fabricius (2000) conclude that SPM is a source of food to an extent, as for *G. retiformis*, but when levels of SPM are too high it acts as a stressor to the coral, as for *P. cylindrica*. They showed that certain SPM concentration did not negatively affect the coral *G. retiformis* but did negatively affect the coral *P. cylindrica*, suggesting that SPM could also be a species-specific stressor.

In conclusion, there are a variety of environmental factors that corals must adapt to when living in shallow and mesophotic environments including light levels and food availability. The corals observed in our study, *M. cavernosa* and *A. agaricites* demonstrate individuality in response to depth, *M. cavernosa* colonies changing its fatty acid profile in the SAFA and PUFA while *A. agaricites* fatty acid profile remained comparable between shallow and mesophotic colonies. Because of the numerous possibilities as to why the fatty acid content was variable in shallow and mesophotic colonies of *M. cavernosa*, there is no definitive answer as to why this variation occurred. Some possibilities as to why fatty acid content varied with *M. cavernosa* include feeding saturation (Anthony & Fabricius, 2000), light levels (Titlyanov et al., 2001), and the new dependence of heterotrophy over translocation in mesophotic ecosystems compared to shallow waters (Clayton Jr. & Lasker, 1982). In contrast, the fatty acid content of *A. agaricites* did not vary with depth. These differences in the potential nutrient and fatty acid acquisition strategies in these coral species highlight the species-specific differences. *M. cavernosa* seemingly needed to increase heterotrophy to survive in mesophotic environments where light levels were low and photosynthates were not as easily obtained as in shallow colonies. This assumption was based on the changing FA content of *M. cavernosa* species growing in shallow and mesophotic environments. In contrast, the FA

content within *A. agaricites* shallow and mesophotic colonies did not change. This result along with literature review could mean that *A. agaricites* did not need to undergo changes in methods of nutrient acquisition to acclimate to the different environmental conditions imposed by increasing depth.

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Appendices

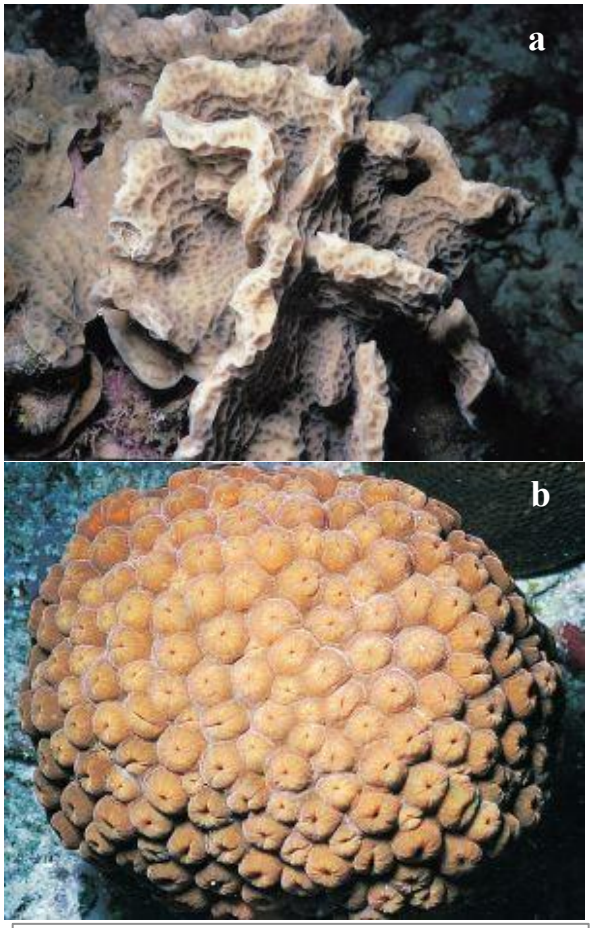

Figure 1. Photographs of the Scleractinian corals *Agaricia agaricites* (a) (Veron, 2013a) and *Montastraea cavernosa* (b) (Veron, 2013b)

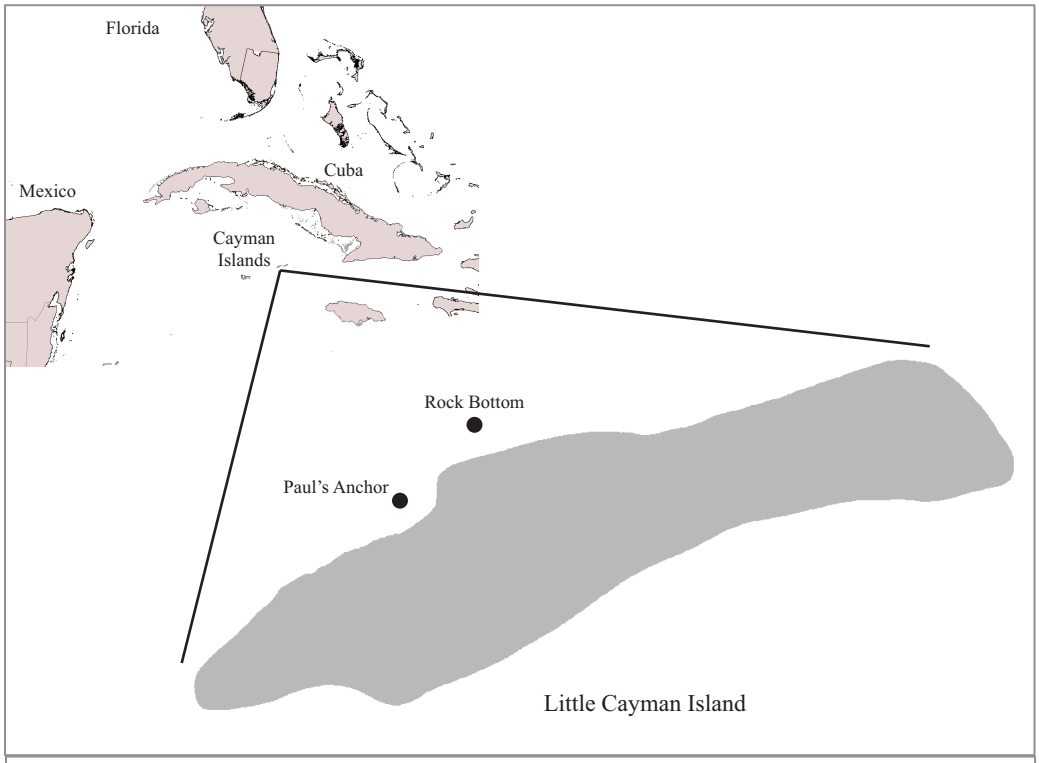

Figure 2. Sampling sight for *M. cavernosa* and *A. agaricites*. Little Cayman Island, Cayman Islands.

Table 1: Fatty acid content of shallow and deep colonies of Scleractinian coral. Values are calculated percent total fatty acid from each species. In addition, relative abundance of saturated fatty acid (SAFA) including C14:0, C16:0, and C18:0; monounsaturated fatty acid (MUFA) including C16:1 ω 9, C16:1 ω 7, C18:1 ω 9, and C18:1 ω 7; and polyunsaturated fatty acid (PUFA) including C18:2 ω 6, C20:4 ω 6, C20:5 ω 3, C20:3 ω 6 , C22:6 ω 3, C22:4 ω 6, C24:5 ω 6, C24:6 ω 3 are shown below.

	M. cavernosa n=5 Shallow		*M. cavernosa* n=9 Deep		*A. agaricites* n=4 Shallow		*A. agaricites* n=8 Deep	
FA	**mean**	**std error**	**mean**	**std error**	**mean**	**std error**	**mean**	**std error**
C14:0	2.9 %	0.2%	2.6%	0.1%	2.0%	0.4%	3.1%	0.3%
C16:0	60%	2.2%	55%	3.1%	41%	2.8%	57%	4.7%
C16:1ω9	0.1%	0.0%	0.3%	0.0%	0.3%	0.2%	0.6%	0.2%
C16:1ω7	2.2%	0.3%	2.6%	0.2%	2.6%	0.6%	4.9%	0.5%
C18:0	18%	1.1%	13%	0.4%	21%	0.8%	11%	0.6%
C18:2ω6	1.4%	0.1%	2.3%	0.3%	2.1%	0.4%	2.1%	0.5%
C18:1ω9	8.0%	0.2%	7.4%	0.4%	5.9%	1.2%	3.2%	0.3%
C18:1ω7	1.7%	0.5%	1.5%	0.1%	1.8%	0.2%	2.4%	0.2%
C20:4ω6	2.0%	0.5%	3.9%	0.9%	7.1%	0.8%	3.5%	1.1%
C20:5ω3	0.9%	0.3%	1.9%	0.4%	1.9%	0.7%	1.7%	0.6%
C20:3ω6	0.3%	0.03%	0.9%	0.2%	0.6%	0.2%	1.0%	0.2%
C22:6ω3	1.3%	0.2%	6.5%	1.7%	6.4%	2.0%	4.8%	1.9%
C22:4ω6	1.2%	0.4%	1.8%	0.4%	5.1%	0.9%	2.1%	0.9%
C24:5ω6	0.2%	0.05%	0.2%	0.04%	1.4%	0.2%	0.8%	0.2%
C24:6ω3	0.3%	0.04%	0.2%	0.03%	0.4%	0.1%	0.6%	0.1%
SAFA	80%	1.5%	71%	3.4%	64%	2.6%	72%	4.8%
MUFA	12%	0.3%	12%	0.5%	11%	1.3%	11%	0.8%
PUFA	7.6%	1.4%	18%	3.5%	25%	3.6%	17%	4.6%

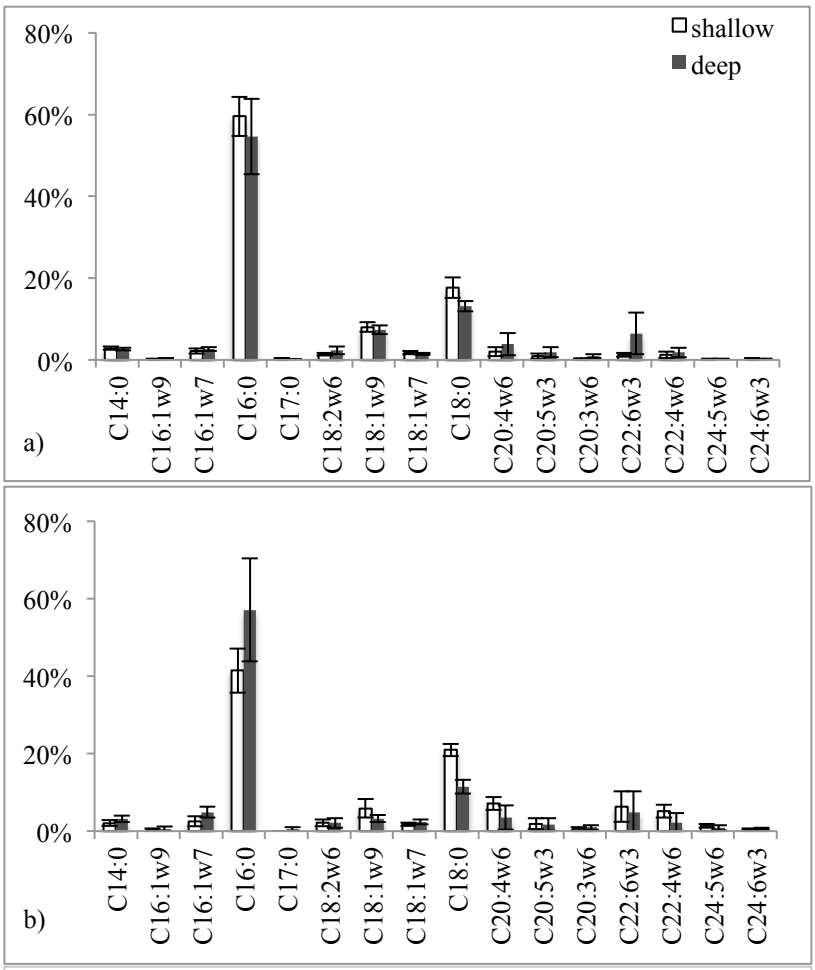

Figure 3: Relative abundance of fatty acid in shallow (white bar) and mesophotic (black bar) colonies of *M. cavernosa* (a) and *A. agaricites* (b).

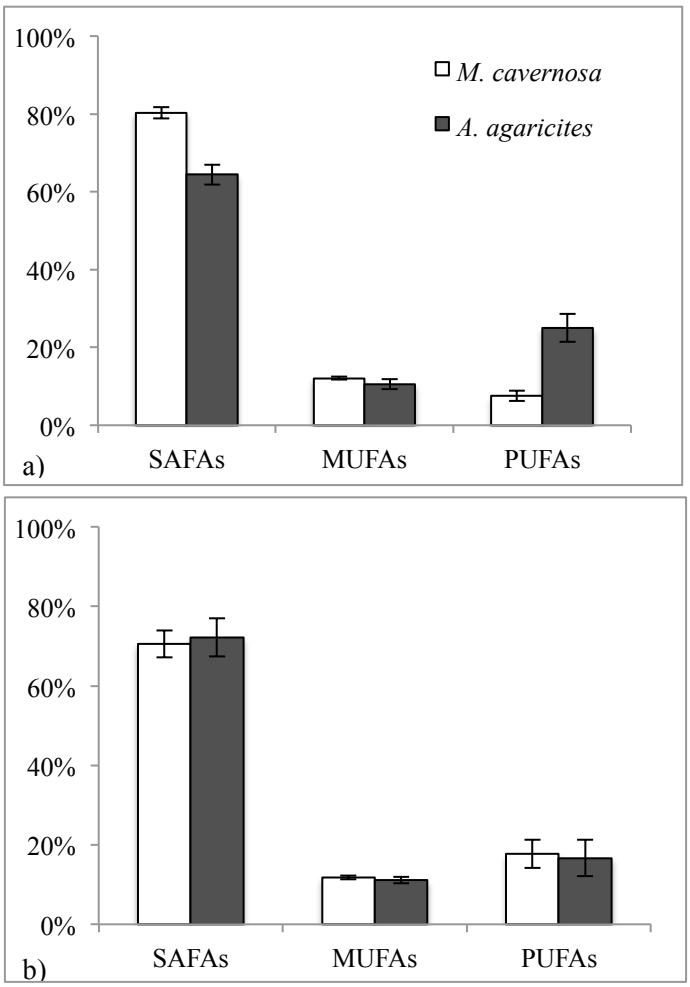

Figure 4. Relative abundance of SAFA, MUFA, and PUFA for *M. cavernosa* and *A. agaricites* of shallow (a) and mesophotic (b) environments. Significant differences observed between shallow colonies of *A. agaricites* and *M. cavernosa* in SAFA content [$t(11)=2.62$ ($p=0.024$)] *M. cavernosa* containing more SAFA than *A. agaricites*; and PUFA content [$t(5)=4.89$ ($p=0.005$)] *M. cavernosa* containing less PUFA than *A. agaricites*. MUFA content was similar in all colonies at all depths.

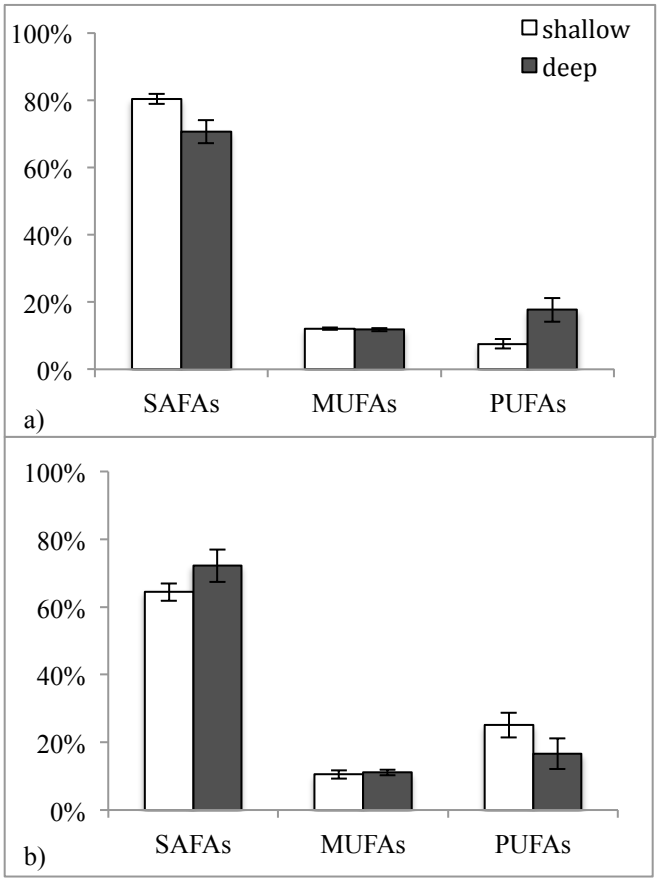

Figure 5: Relative abundance of SAFA, MUFA, and PUFA percent total fatty acid in shallow (white bar) and deep (black bar) colonies of *M. cavernosa* (a) and *A. agaricites* (b).